Identification and Pathogenicity of *Rhizoctonia* spp. Isolated from Apple Roots and Orchard Soils

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ABSTRACT

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Rhizoctonia spp. were isolated from the roots of apple trees and associated soil collected in orchards located near Moxee, Quincy, East Wenatchee, and Wenatchee, WA. The anastomosis groups (AGs) of *Rhizoctonia* spp. isolated from apple were determined by hyphal anastomosis with tester strains on 2% water agar and, where warranted, sequence analysis of the rDNA internal transcribed spacer region and restriction analysis of an amplified fragment from the 28S ribosomal RNA gene were used to corroborate these identifications. The dominant AG of *R. solani* isolated from the Moxee and East Wenatchee orchards were AG 5 and

AG 6, respectively. Binucleate *Rhizoctonia* spp. were recovered from apple roots at three of four orchards surveyed and included isolates of AG-A, -G, -I, -J, and -Q. In artificial inoculations, isolates of *R. solani* AG 5 and AG 6 caused extensive root rot and death of 2- to 20-week-old apple transplants, providing evidence that isolates of *R. solani* AG 6 can be highly virulent and do not merely exist as saprophytes. The effect of binucleate *Rhizoctonia* spp. on growth of apple seedlings was isolate-dependent and ranged from growth enhancement to severe root rot. *R. solani* AG 5 and AG 6 were isolated from stunted trees, but not healthy trees, in an orchard near Moxee, WA, that exhibited severe symptoms of apple replant disease, suggesting that *R. solani* may have a role in this disease complex.

Rhizoctonia solani is a ubiquitous soilborne pathogen capable of causing severe economic damage to a wide variety of plant species. Many tree species are susceptible to *R. solani* (9), and this pathogen is of primary importance in seedbeds and nurseries, where it is a common cause of damping-off and root rot of seedlings. In addition, *R. solani* has been isolated from roots of trees grown in landscape environments and orchards, but has not been shown to be an important root pathogen of older trees. There are few reports of this fungus causing diseases of orchard trees (2,13,22). Although *R. solani* has been reported to cause a disease on apple (1,7,8), the study by Burr et al. (2) of a severe root rot of apple seedlings in commercial nurseries in New York appears to be the only study to document the potential economic significance of this pathogen on apple.

R. solani is a taxonomic species composed of morphologically similar fungi isolated by hyphal incompatibility into at least 12 anastomosis groups (AGs). There are no reports concerning the AGs of *R. solani* that are capable of infecting apple. Likewise, the relative importance of different AGs in causing disease on apple, or the possible involvement of other species of *Rhizoctonia* in root rot of apple either in nursery or orchard environments, has yet to be examined. Given the variability among species and AGs of *Rhizoctonia* in many significant characteristics, including sensitivity to chemical (15,27) and biological control agents (14,20), successful management of *Rhizoctonia* root rot of apple will require a more complete understanding of the causal fungus. The objective of this study was to characterize isolates of *Rhizoctonia* spp. associated with apple roots and investigate the possible role of *Rhizoctonia* spp. in a root rot disease observed in new and established apple orchards in Washington state. A preliminary report of this work has been published (19).

MATERIALS AND METHODS

Isolation of *Rhizoctonia* spp. Soil and root samples were collected from the Washington State University/USDA-ARS Columbia View Experimental Orchard (CV orchard), located 20 km north of East Wenatchee, WA; the Wenatchee Valley College Experimental Orchard (WVC orchard), located 1.5 km north of Wenatchee, WA; and commercial orchards near Moxee (DR orchard) and Quincy (CW orchard), WA. The study site at the DR orchard was prepared for renovation in November 1994 by removal of trees and burning of slash on site. The site was not fumigated prior to replanting the site in April 1995 with five rows (6.1 × 2.4-m spacing) of the apple cultivar Gala on M26 rootstock, with four rows planted directly into the rows of the previous orchard. The fifth row was established on the west edge of the block in soil that had not previously been planted to apple. Soil and root samples were obtained from a 5-year-old planting of 'Braeburn' on M7 rootstock and a 7-year-old planting of 'Gala' on M9 rootstock at the CW and WVC orchards, respectively. Samples were collected from a 4-year-old planting containing various scion cultivars on M26 rootstock, a 7-year-old planting of 'Red Delicious' on M111 rootstock, and a 26-year-old planting of 'Red Delicious' on seedling rootstock at the CV orchard.

Soil and root samples were randomly collected from 30 individual trees at the DR orchard site on 12 September and 9 November 1995 and 10 January 1996, and 10 sites within each sampled block at the CV, CW, and WVC orchards between 21 September 1995 and 30 May 1996. Soil samples (approximately 750 cm³) were collected from within the root zone at a depth of 10 to 30 cm below the surface.

Isolation of *Rhizoctonia* spp. from field-infected apple tissue was conducted by washing roots with tap water to remove adhering soil particles and plating root segments (0.5 to 1.0 cm in length) on 1.5% water agar amended with kanamycin sulfate (75 µg/ml) or ampicillin (100 µg/ml). Baiting methods were used to isolate *Rhizoctonia* spp. from orchard soils. Apple seeds ('Gala') were surface-sterilized in a 10% solution of commercial bleach (5.25% sodium hypochlorite) and rinsed for 30 min under running

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tap water. Seeds were dusted with captan 50WP and stratified for germination by placing seed in a plastic bag with moistened paper towels at 4°C for 6 to 8 weeks. Germinated seeds were sown into a sterile peat moss/perlite mix and incubated at 24°C with a 12-h photoperiod. Conical plastic tubes (20.5 × 4 cm [top diameter]; Stuewe & Sons, Inc., Corvallis, OR) were filled with a 50-cm³ layer of vermiculite and topped with a 50-cm³ layer of orchard soil. One 21-day-old apple seedling was transplanted into each tube, with a total of seven tubes planted for each soil sample. Seedlings were grown in controlled environment growth chambers at 18°C with a 12-h photoperiod. Seedlings were harvested after 14 days, and roots were washed under a high pressure stream of water. Excised root segments were plated onto water agar as described above and incubated at 20 to 23°C for 72 h, and hyphae emanating from root segments were examined using a light microscope (Olympus BH2 series; Olympus Optical Co. Ltd., Japan). *Rhizoctonia*-like hyphae were transferred to fresh 1/5-strength potato dextrose agar (PDA) (Difco Laboratories, Detroit) medium amended with ampicillin (100 µg/ml) and stored at 4°C.

Anastomosis typing of *Rhizoctonia* spp. isolates. Nuclear condition of isolates of *Rhizoctonia* spp. was determined by distributing a wetting solution (Tween 20) over mycelium on a culture plate, staining hyphae with 0.5% aniline blue in lactophenol (10), and observing hyphae microscopically. Anastomosis typing of *Rhizoctonia* spp. isolates was conducted on 2% water agar in petri dishes (26), with each unknown isolate paired with an isolate of known anastomosis grouping (Table 1). Confirmation of genetic affinities for some isolates of *R. solani* was conducted by comparison of the nucleotide sequences of the internal transcribed spacer (ITS) 1 region from tester strains with that of unknown isolates. The ITS nucleotide sequences for isolates of *R. solani* AGs 1 to 6 and 8 were determined previously (21). Genomic DNA was extracted from each isolate of *Rhizoctonia* as described previously (21). The primers ITS 1 and ITS 4 (32) were used in polymerase chain reaction (PCR) reactions with genomic DNA from isolates of *Rhizoctonia* to amplify a region that spans from the 3' end of the 18S rRNA gene to the 5' end of the 28S rRNA gene and includes the 5.8S rRNA gene and the two ITS regions (ITS 1 and ITS 2). Amplifications of the ITS sequence were conducted in a DNA Thermal Cycler 480 (Perkin-Elmer Corp., Norwalk, CT) using reaction conditions described by Mazzola et al. (21). The resulting PCR products were cloned into the vector pCRII (TA cloning kit; Invitrogen Corp., San Diego, CA) and transformed into *Escherichia coli* INVαF' using the protocol provided by the manufacturer. The ITS 1 region from *Rhizoctonia* spp. was sequenced using an ABI Prism 377 automated DNA sequencer (Perkin-Elmer Corp.). Restriction analysis of the 28S rRNA gene was conducted to corroborate results obtained from anastomosis tests on water agar and sequencing of the ITS region that indicated that certain isolates of *R. solani* from apple belonged to AG 6. A portion of the 28S rRNA gene was amplified from genomic DNA of isolates of *Rhizoctonia* using the primers LR0R and LR7 (30) in PCR reactions. The reaction conditions were those described by Cubeta et al. (5) for amplification of this region from binucleate *Rhizoctonia* spp. The amplified PCR products were digested with *Hha*I, *Hpa*II, or *Taq*I, and DNA fragments were separated in a 1.5% agarose gel in Tris-borate-EDTA buffer. The gel was stained with ethidium bromide, and the DNA fragments were viewed using a UV transilluminator.

Pathogenicity assays. The ability of isolates of *Rhizoctonia* to cause root rot of apple were determined in assays using transplants of varying age. Seeds of the cultivar Gala were sown in plastic flats containing pasteurized (exposed to steam at 97°C for 90 min) peat moss/perlite growth medium and placed in growth chambers at 24°C with a 12-h photoperiod for up to 21 days. When larger plants were required, 21-day-old seedlings were transplanted into 16-cm-diameter plastic pots containing pasteurized Burch sandy loam from the CV orchard and grown in the

greenhouse at 20°C without supplemental lighting. Pathogenicity assays were performed in natural and pasteurized Willis silt loam (WSL) collected from the DR orchard. Inoculum of *Rhizoctonia* was prepared by growing isolates individually in 500-ml Erlenmeyer flasks containing 250 ml of oat bran and 80 ml of distilled water. The flasks were sterilized on two consecutive days for 90 min and inoculated with a culture of *Rhizoctonia* growing on 1/5-strength PDA. Flasks were incubated at 20°C for 14 days, and then inoculum was air-dried under a laminar flow hood. Oat bran inoculum was added to soil at a rate of 0.25% (wt/wt), and soils were planted after 24 h of incubation at 20 to 23°C. Assays with 2- and 3-week-old seedlings were conducted in conetainers, with a single seedling planted per tube and 14 replicates per isolate of *Rhizoctonia*. Pathogenicity assays using 7- and 12-week-old trees were performed in 16-cm-diameter plastic pots with three seedlings per pot, and each treatment was replicated four times. Assays conducted with 20-week-old seedlings were performed in 16-cm-diameter pots with one tree per pot, and each treatment was replicated seven times. In each assay, seedlings grown in pasteurized or natural soil without added inoculum of *Rhizoctonia* spp. served as controls. Plants were placed in environmental growth chambers at 18°C with a 12-h photoperiod. Assays using 2- or 3-week-old seedlings were harvested after 2 weeks, and those conducted with 7- and 12-week-old trees were harvested 3 and 6 weeks after transplanting, respectively. Shoot and root weight were determined at harvest. When 20-week-old transplants were used in

TABLE 1. Anastomosis group (AG) and origin of tester isolates of *Rhizoctonia* spp. used in this study

Isolate	AG	Host or habitat	Source ^a
<i>Rhizoctonia solani</i>			
R. shi-2	1-1B	... ^b	Cook
F-2	1-1C	...	Cook
H-24	2-1	...	Cook
455-11	2-2	...	Cook
ST-9	3	...	Cook
6-3-6	4	<i>Triticum aestivum</i>	Cook
2-5-5	4	<i>T. aestivum</i>	Cook
D-1	5	<i>T. aestivum</i>	Cook
SO-2-1	6	...	Cook
TSH1-1	6	...	Cook
C-1	8	<i>Hordeum vulgare</i>	Cook
2-10-2	10	<i>H. vulgare</i>	Cook
Binucleate <i>Rhizoctonia</i> spp.			
C-662	A	Soil	Cubeta
BN4	A	Soil	Cubeta
C-455	Bb	<i>Oryzae sativa</i>	Cubeta
SIR-2	B(o)	<i>Ipomoea batatas</i>	Cubeta
70B	C	Soil	Cubeta
C-610	D	...	Cubeta
BN1	D	<i>Agrostis</i> sp.	Cubeta
1-1b-9	E	<i>T. aestivum</i>	Cook
BN31	E	<i>Arachis hypogaea</i>	Cubeta
SIR-1	F	<i>I. batatas</i>	Cubeta
BN38	F	<i>Glycine max</i>	Cubeta
C-653	G	...	Cubeta
STC-9	H	Soil	Cubeta
AV-2	I	<i>Artemisia</i> sp.	Cook
184	J	...	Cubeta
FA52909	K	...	Cubeta
76146	L	...	Caesar
TC1	N	...	Caesar
76150	P	...	Caesar
C-620	Q	Soil	Cubeta
TC2	R	...	Caesar
BN37	R	<i>Cucumis sativum</i>	Cubeta
TC3	S	...	Caesar
BN22	S	<i>Pittosporum</i> sp.	Cubeta

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^b Unknown.

pathogenicity assays, the weight of each tree was determined prior to planting and again after 6 weeks. The increase in plant biomass of trees grown in soil infested with *R. solani* was compared with that of trees grown in noninfested soil.

RESULTS

Isolation and identification of *Rhizoctonia* spp. from apple.

A total of 87 isolates of *Rhizoctonia* were recovered from apple roots or baited from orchard soil. All isolates came from different field-infested trees or different seedlings planted in orchard soil samples. Of these, 32 isolates were multinucleate and 55 were binucleate. The composition of *Rhizoctonia* isolates recovered from apple roots and associated soil varied among the surveyed orchards. *R. solani* was isolated from the DR and CV orchards, but not the WVC and CW orchards. Based on hyphal anastomosis with known tester isolates, all isolates of *R. solani* from the CV orchard were classified as AG 6, while the population recovered from the DR orchard consisted of 18 isolates belonging to AG 5

and three isolates identified as AG 6. The ITS 1 nucleotide sequence of isolate E22-4 from the DR orchard possessed 98% sequence identity with the corresponding fragment from *R. solani* AG 5 tester isolate D-1, while isolate CV-B had 92% sequence identity in the same region of DNA with *R. solani* AG 6 tester isolates SO-2-1 and TSH1-1. The restriction pattern obtained after digestion of the amplified 28S rRNA region from isolates CV-B and CV-D with either *TaqI* or *HhaI* (data not shown) was identical to that of isolate TSH1-1, but also was the same as that observed for isolates of AG 8 and AG BI. The restriction pattern obtained with *HpaII* for isolates of AG 6 was distinctive and allowed for the separation of isolates of AG 6 from AG 8 and AG BI isolates (Fig. 1).

Binucleate *Rhizoctonia* spp. were isolated from apple roots or soil obtained from the CW, DR, and WVC orchards. Restriction analysis of the rDNA fragment indicated that the population of binucleate *Rhizoctonia* spp. recovered from both the DR and CW orchards was composed of at least five different groups, but that all binucleate *Rhizoctonia* isolates from the WVC orchard represented a single group. Based on anastomosis with tester isolates, all binucleate *Rhizoctonia* spp. from the WVC orchard belonged to AG-I, the collection from the CW orchard contained isolates of AG-G, -I, and -J, and those from the DR orchard were identified as AG-A, -G, and -Q. Restriction patterns obtained by *HpaII* digestion of the amplified 28S rDNA fragment from AG-A and AG-G isolates from apple were similar to those of tester isolates, but those of AG-I, -J, and -Q were different from the tester strains (Fig. 2). Polymorphisms were also observed in the restriction patterns between AG-I isolates from the WVC orchard and those from the CW orchard (Fig. 2). Isolates from the DR and CW orchards representing at least two different groups could not be identified to an AG based on restriction analysis of the rDNA fragment or anastomosis with the known tester isolates used in this study. Based on restriction fragment length polymorphisms, one group from the DR orchard consisted of isolates B41-2, C41-1,

Fig. 1. Restriction enzyme digestion (*HpaII*) of a polymerase chain reaction-amplified portion of 25S rDNA using the primers LR0R and LR7 and purified DNA from isolates of *Rhizoctonia solani*. Lanes 1 and 12, 1-kb DNA ladder; lane 2, AG 1 isolate F-2; lane 3, AG 2 isolate H-24; lane 4, AG 3 isolate ST-9; lane 5, AG 4 isolate 6-3-6; lane 6, AG 5 isolate D-1; lane 7, AG 6 isolate CV-B; lane 8, AG 6 isolate CV-D; lane 9, AG 6 isolate TSH1-1; lane 10, AG 8 isolate C-1; and lane 11, AG BI isolate Ts2-4.

Fig. 2. Restriction enzyme digestion (*HpaII*) of a polymerase chain reaction-amplified portion of 25S rDNA using the primers LR0R and LR7 and purified DNA from isolates of binucleate *Rhizoctonia* spp. Lane 1, AG-A isolate C-662; lane 2, AG-A isolate B21-3; lane 3, AG-G isolate C-653; lane 4, AG-G isolate E39-2; lane 5, AG-I isolate AV-2; lane 6, AG-I isolate WVC-8; lane 7, AG-I isolate CW1-7; lane 8, AG-J isolate 184; lane 9, AG-J isolate CW2-2; lane 10, AG-Q isolate C-620; lane 11, AG-Q isolate E3-2; and lane 12, 1-kb DNA ladder.

TABLE 2. Effect of *Rhizoctonia* spp. isolated from apple trees at the DR^a and CV^b orchards on growth of 3-week-old apple ('Gala') seedling transplants in natural Willis silt loam

Isolate	AG ^c	Trial 1		Trial 2	
		Shoot wt (mg)	Root wt (mg)	Shoot wt (mg)	Root wt (mg)
Control		433	248	680	266
C13-2	A	433	298	516	227
B21-3	A	365	235	561	336
B6-1	G	396	235	538	220
B6-2	G	387	195	592	298
B46-1	G	394	191	514	310
E39-2	G	361	160	412	161
E3-2	Q	200	112	425	144
B41-2	UK1	436	264	512	271
C41-1	UK1	359	177	481	230
C46-1	UK1	451	278	557	229
D44-2	UK2	481	369	574	358
D44-3	UK2	461	267	613	257
E44-1	UK2	441	329	626	349
B20-1	5	246	86	494	169
B32-1	5	332	158	473	186
B41-1	5	175	126	408	123
E22-4	5	298	127	352	142
5-103	5	292	131	459	113
5-104	5	303	103	359	98
CV-B	6	298	122	211	59
D20-1	6	246	86	187	25
LSD (<i>P</i> = 0.05)		94	79	132	77

^a Commercial orchard near Moxee, WA.
^b Washington State University/USDA-ARS Columbia View Experimental Orchard, located 20 km north of East Wenatchee, WA.
^c UK signifies that the anastomosis group (AG) is not known, but all isolates with the same number following this designation belong to the same AG based on anastomosis tests on water agar.

and C46-1, and a second group contained isolates D44-2, D44-3, and E44-1. The unclassified groups from the CW orchard consisted of isolates CW1-8 and CW2-1, and a second group contained isolates CW1-1, CW1-2, and CW1-6.

Pathogenicity of *Rhizoctonia* spp. on apple. The ability of *Rhizoctonia* spp. to cause root rot of apple was evaluated using 2-, 3-, 7-, 12-, or 20-week-old transplants. Initial assays were conducted using 2-week-old apple seedlings that were transplanted into pasteurized WSL infested with a single isolate of *R. solani* AG 5 or *R. solani* AG 6. The isolates of *R. solani* AG 5 used in this experiment were E22-1, E22-2, E22-3, and E22-4, while those of AG 6 were CV-A, CV-B, CV-C, and CV-D. These isolates were chosen for evaluation as they represented the dominant species of *Rhizoctonia* isolated from the DR and CV orchards, respectively. Extensive root rot of seedlings was apparent within 72 h, and all plants grown in infested soil had wilted within 7 days of transplanting, while those seedlings transplanted into noninfested soil exhibited no symptoms of root rot or wilt. Root segments from three seedlings for each soil treatment were plated onto 1.5% water agar amended with ampicillin. *R. solani* was reisolated from each seedling grown in artificially infested soil, but was not recovered from seedlings grown in noninfested pasteurized soil.

Of the 87 isolates of *Rhizoctonia* from apple roots or orchard soil, 34 isolates were evaluated in pathogenicity assays using 3-week-old apple seedlings in natural WSL. Isolates evaluated in these assays were chosen so that all known AGs, as well as groups that were genetically distinct but could not be placed into an AG, were represented. Eleven of the 34 isolates of *Rhizoctonia* caused root rot of apple, resulting in significant reductions in both shoot and root biomass (Table 2). With the exception of isolate E39-2, all isolates of *Rhizoctonia* that were pathogenic to apple also caused seedling mortality, which was 50% or greater in soil infested with isolate B20-1, CV-B, D20-1, E3-2, E22-4, 5-103, or 5-104. Isolates of *Rhizoctonia* that were pathogenic on apple were identified as *R. solani* AG 5 or AG 6, except isolates E3-2, E39-2, and WVC-8 (Table 3), which are binucleate *Rhizoctonia* spp. belonging to AG-Q, -G, and -I, respectively. Isolates D44-2 and E44-1 significantly enhanced root biomass of seedlings grown in WSL (Table 2). The remaining isolates of binucleate *Rhizoctonia* spp. had no apparent effect on the growth of 3-week-old apple seedlings (Tables 2 and 3).

TABLE 3. Effect of *Rhizoctonia* spp. isolated from apple trees at the CW^a and WVC^b orchards on growth of 3-week-old apple ('Gala') seedling transplants in natural Willis silt loam

Isolate	AG ^c	Trial 1		Trial 2	
		Shoot wt (mg)	Root wt (mg)	Shoot wt (mg)	Root wt (mg)
Control		423	205	425	147
CW-1	G	348	166	425	121
CW-2	G	345	151	342	125
CW1-7	I	332	127	470	134
CW1-9	I	308	131	414	127
CW1-10	I	383	162	429	147
CW3-1	I	347	139	498	159
WVC-8	I	206	58	263	66
CW2-2	J	300	129	481	167
CW1-1	UK3	328	153	419	152
CW1-2	UK3	309	135	434	165
CW1-6	UK3	325	137	453	174
CW1-8	UK4	348	178	401	133
CW2-1	UK4	310	151	424	137
LSD ($P = 0.05$)		163	117	107	71

^a Commercial orchard near Quincy, WA.

^b Wenatchee Valley College Experimental Orchard, located 1.5 km north of Wenatchee, WA.

^c UK signifies that the anastomosis group (AG) is not known, but all isolates with the same number following this designation belong to the same AG based on anastomosis tests on water agar.

Among the isolates of *Rhizoctonia* spp. examined, *R. solani* AG 5 isolates E22-4 and 5-104, and *R. solani* AG 6 isolate CV-B, consistently caused severe damage to apple seedlings that were 14 or 21 days old at the time of transplanting into infested soil. Therefore, these isolates were used in assays to determine the susceptibility of 7-week-old apple transplants to *R. solani*. In the absence of added inoculum of *R. solani*, shoot and root weights were significantly ($P \leq 0.001$) larger for plants grown in pasteurized than in natural DR orchard soil (Table 4). Both *R. solani* AG 5 and *R. solani* AG 6 caused root rot of 7-week-old apple seedlings in pasteurized soil, resulting in significant reductions in root and shoot weight (Table 4). In natural soil, all three isolates of *R. solani* caused root rot of apple, resulting in lower root biomass, but only isolates CV-B and 5-104 caused a significant reduction in shoot weight. Seedling mortality was 25 and 42% for plants grown in natural and pasteurized soil, respectively, infested with *R. solani*, but seedling mortality was not observed in noninfested soil.

Pasteurization had a significant ($P \leq 0.001$) positive effect on growth of 12-week-old apple transplants in noninfested soil from the DR orchard (Table 5). In pasteurized soil, all three isolates of *R. solani* significantly reduced the biomass of apple transplants. In natural soil, isolates CV-B and 5-104 each caused root rot of 12-week-old transplants, but isolate E22-4 did not cause a significant reduction in plant biomass. All transplants grown in noninfested soil or soil infested with isolate E22-4 were alive at harvest, but mortality was observed for 16 and 25% of transplants grown in soil infested with isolates CV-B and 5-104, respectively.

Twenty-week-old apple trees transplanted into *R. solani*-infested soil were harvested 6 weeks after initial exposure to the pathogen. The increase in plant biomass during the 6-week period for trees grown in soil infested with *R. solani* 5-104 and CV-B was 31 and 15%, respectively, of that obtained in noninfested soil, and both isolates caused tree mortality.

DISCUSSION

Isolates of *Rhizoctonia* spp. were recovered from the roots of recently established orchard transplants exhibiting symptoms of apple replant disease, from mature apple trees that ranged from 4 to 26 years of age, and associated orchard soil. The collection of *Rhizoctonia* spp. obtained from the surveyed orchards consisted of a diverse group of multinucleate and binucleate species. Isolates of *R. solani* AG 5 and AG 6 were recovered from field-infected trees in the DR orchard, while all isolates of *R. solani* from the

TABLE 4. Effect of *Rhizoctonia solani* anastomosis group (AG) 5 isolates E22-4 and 5-104 and AG 6 isolate CV-B on growth of 7-week-old apple ('Gala') transplants

Isolate	Natural soil		Pasteurized soil	
	Shoot wt (g)	Root wt (g)	Shoot wt (g)	Root wt (g)
Control	0.87	0.70	1.09	1.15
E22-4	0.78	0.48	0.78	0.25
5-104	0.59	0.34	0.72	0.28
CV-B	0.63	0.33	0.71	0.30
LSD ($P = 0.05$)	0.21	0.16	0.17	0.15

TABLE 5. Effect of *Rhizoctonia solani* anastomosis group (AG) 5 isolates E22-4 and 5-104 and AG 6 isolate CV-B on growth of 12-week-old apple ('Gala') transplants

Isolate	Natural soil		Pasteurized soil	
	Shoot wt (g)	Root wt (g)	Shoot wt (g)	Root wt (g)
Control	1.99	0.96	2.24	1.73
E22-4	1.62	0.79	1.65	0.61
5-104	1.40	0.35	1.22	0.44
CV-B	1.37	0.38	1.42	0.42
LSD ($P = 0.05$)	0.36	0.27	0.46	0.32

CV orchard were identified as AG 6. Isolates from both AG 5 and AG 6 were aggressive pathogens causing severe root rot and ultimately death of apple transplants regardless of plant age at the time of exposure to the pathogen.

The AGs of *R. solani* isolated from apple are rather unusual, as AG 5 has been reported to be weakly virulent relative to other AGs on several plant hosts (3,28,33), and isolates from AG 6 have not been considered to be plant pathogens (28) or only weakly virulent (16). Because the preliminary identification in hyphal anastomosis tests of certain *R. solani* isolates from apple as AG 6 was unexpected, and members of AG 6 are known to anastomose with isolates from AG 8, AG BI, and AG-F at low frequency (28), sequence analysis of the ITS region and restriction analysis of an amplified region of the 28S rRNA gene were conducted to verify initial findings. Within an AG of *R. solani*, sequence identity in ITS 1 was greater than 90%, while sequence identity between AGs ranged from 60 to 80% (20; M. Mazzola, unpublished data). The ITS 1 sequence from CV-B possessed greater than 90% identity with the corresponding sequence from a tester strain of AG 6. Likewise, the restriction pattern obtained by digestion of the amplified fragment of rDNA from isolates CV-B and CV-D with *Hpa*II was identical to that for a known isolate of AG 6, but different from that of any other *R. solani* AG tested. Both of these analyses supported the original determination that these isolates belong to AG 6. Thus, the results of this study demonstrate that isolates of *R. solani* AG 6 do not simply exist as saprophytes, but can be severe pathogens on certain plant species.

R. solani isolates CV-B, E22-4, and 5-104 caused similar damage to 7- or 12-week-old apple seedlings when pathogenicity assays were conducted in pasteurized soil. However, isolates CV-B and 5-104, but not isolate E22-4, caused significant reductions in plant growth when assays were conducted in natural WSL from the DR orchard. DR orchard soil contained isolates of *R. solani*, *Pythium ultimum*, *Phytophthora cactorum*, and *Cylindrocarpon* spp. that were pathogenic to apple (M. Mazzola and F. Martin, unpublished data). In assays to examine the pathogenicity of *Rhizoctonia* spp., these fungal pathogens caused extensive damage to apple transplants in noninfested WSL, making it difficult to assess the relative contribution of isolate E22-4 to the root disease observed in these tests. These findings could also indicate that isolate E22-4 is a less capable rhizosphere colonist relative to isolates CV-B and 5-104 in competition with the indigenous soil microflora.

Binucleate *Rhizoctonia* spp. are known to form diverse plant associations ranging from symbiotic, the case with *R. repens*, which forms mycorrhizal associations with orchids (6,31), to pathogenic, as is the case for *R. cerealis*, which causes sharp eyespot of wheat (17). Isolates of binucleate *Rhizoctonia* spp. recovered from apple roots or orchard soil were diverse both in their effect on apple seedling growth and in anastomosis grouping. The effect of binucleate *Rhizoctonia* spp. recovered in this study on growth of apple was isolate-specific and ranged from beneficial to deleterious. However, as observed for binucleate *Rhizoctonia* spp. on other plant hosts (11,28,33), the majority of isolates were avirulent on apple. Binucleate *Rhizoctonia* spp. isolated from apple represented five known AGs, AG-A, -G, -I, -J, and -Q, and at least two additional *Rhizoctonia*-like groups that did not anastomose with any tester isolates used in this study. Isolates that were pathogenic to apple belonged to AG-G, -Q, and -I, but not all isolates from these AGs caused root rot of apple in growth chamber experiments at 18°C. Pathogenic isolates of *Rhizoctonia* spp. AG-G have been isolated from a number of plant species (28); isolates of AG-I have been reported to cause a root rot of strawberry (18) and AG-Q causes a disease of turf grass (25), but isolates from these AGs have not previously been isolated from apple. The two binucleate *Rhizoctonia*-like isolates that enhanced growth of apple could not be identified to an AG. However, these isolates appear to be of the same AG, as hyphal anastomosis was observed between the two isolates in agar plate assays and digestion of the amplified rDNA gene fragment for these

isolates resulted in identical restriction patterns that were different from all other binucleate *Rhizoctonia* isolates tested.

The prevalence of binucleate and multinucleate *Rhizoctonia* spp. cultures from agroecosystems appears to be crop-specific. Binucleate species made up from 5 to 25% of cultures of *Rhizoctonia* isolated from sugar beets or associated field soil (11,23,33). Binucleate *Rhizoctonia* spp. recovered from roots and stems of soybean accounted for less than 5% of the total population of *Rhizoctonia* isolated from this host (24). In contrast, binucleate *Rhizoctonia* spp. represented approximately 65% of the total collection of *Rhizoctonia* isolated from apple in this study and composed approximately 50% of the population of *Rhizoctonia* spp. recovered from Norway spruce seedlings exhibiting root dieback symptoms in Finland (12). *R. solani* was isolated infrequently from strawberry in comparison with binucleate *Rhizoctonia* spp., with isolates from AG-A, -G, and -I representing approximately 90% of the *Rhizoctonia* population recovered from this host (18).

Cubeta et al. (5) were able to consistently differentiate 13 of 21 binucleate *Rhizoctonia* AGs by restriction analysis of PCR-amplified rDNA. Using the same primers and restriction enzymes in this study, isolates of AG-A and AG-G isolated from apple produced the same restriction patterns as the tester strains for these AGs. However, isolates of AG-I, -J, and -Q from apple produced different restriction patterns than were observed for the tester strains, as did isolates of AG-I from different orchards. These findings point to the inherent level of genetic diversity present among isolates of *Rhizoctonia* spp. within AGs and the potential problems associated with the development of molecular markers for the specific and consistent identification of such a genetically diverse population.

Apple replant disease has been attributed to a variety of biotic and abiotic factors including nematodes, bacteria, fungi, heavy metal contamination, and soil physical properties among others (29). Although numerous fungi have been implicated in this disease phenomenon, there has only been passing mention of the possible role of *Rhizoctonia* spp. in the etiology of apple replant disease. In a histological study of apple roots from trees exhibiting symptoms of replant disease, Caruso et al. (4) observed hyphae of *Rhizoctonia*, but there were no attempts to isolate the fungus and assess its pathogenicity on apple. It was suggested that *R. solani* might be involved in nonspecific apple replant problems in New York (2). In the current study, pasteurization of soil from either the CV or DR orchard resulted in significant improvements in plant growth, and growth of apple in pasteurized soil was equivalent to that obtained in previously unplanted soil from the same site (M. Mazzola, unpublished data), indicating that a replant problem existed at these sites. *R. solani* was only isolated from the roots of severely stunted trees in the recently planted block in the DR orchard and was not isolated from any healthy trees at this site. All of these isolates were pathogenic to apple in growth chamber experiments, causing extensive root rot and tree death. These results suggest that *R. solani* can contribute to the death of young trees in newly established orchards, and studies are in progress to further clarify the role of *R. solani* in apple replant disease.

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